PART A: PROJECT INFORMATION

A1. TITLE OF THE STUDY IN THAI AND ENGLISH

English: A clinical study to assess the safety and feasibility of controlled blood-stage *Plasmodium vivax* human malaria infection through experimental inoculation of cryopreserved infected erythrocytes in healthy Thai adults

Study Code: MAL21001

Trial Registry Name: ClinicalTrials.gov; ClinicalTrials.gov ID: NCT05071079

A2. RESEARCH PROPOSAL VERSION DATE: v.6.0, dated 27 April 2022

Type of submission
- ☐ Initial review (first time submission)
- ☐ Revision according to TMEC suggestions No…
- ☑ Amendment No. ….3…

A3. PRINCIPAL INVESTIGATOR NAME: Professor Nicholas Day and Research Professor Dr. Jetsumon Sattabongkot Prachumsri

- ☑ **Faculty Staff** (Go to A4.1)
- ☐ **Student, ID**……..… (Please check the following, and go to A4.2)
  - ☐ Research for Thesis
    - ☐ M.Sc. (Trop. Med.)
    - ☐ Ph.D. (Trop. Med.)
    - ☐ Ph.D. (Clin. Trop. Med.)
  - ☐ Research for Thematic Paper
    - ☐ M.C.T.M.
    - ☐ M.C.T.M. (T.P.)
    - ☐ Other, specify ……
- ☑ **Other** Mahidol-Oxford Tropical Medicine Research Unit (MORU) Staff (Please specify, and go to A4.1)

A4. LIST NAME, AFFILIATION AND CONTACT DETAILS OF ALL INVESTIGATORS

A4.1 For Faculty staff and other

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A4.2 For students: Not applicable

Name	Position	Contact address	E-mail address
	Principal Investigator		
	Accountable Investigator (Student/Advisor)		
	Advisor		
	Co-advisor		
	Co-advisor		

A5. RESPONSIBILITY OF PI

☑ Whole study
☐ Partial responsibility

A6. NUMBER OF YOUR ONGOING RESEARCH PROJECT(S)

1) Professor Nicholas Day: 5 studies
2) Research Professor Dr. Jetsumon Sattabongkot Prachumsri: 8 studies

A7. CONTACT PERSON

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A8. NATURE OF THE STUDY- STUDY INVOLVING SPECIMEN COLLECTION

- ☑ Clinical trial phase…-…/ (Intervention study)
- ☐ Bioequivalence/ pharmacokinetic drug study
- ☐ Prospective epidemiological research
- ☐ Laboratory study

A9. IS THE PROJECTS A SINGLE CENTER OR MULTI-CENTER?

- ☑ Single center
- ☐ Multicenter (within Thailand)
  Please specify the study sites …….....
- ☐ Multicenter (International)
  Please specify the study sites …….....

A10. PROJECT SUMMARY IN THAI AND ENGLISH

A10.1 Full protocol summary in English (not more than 1,000 words per language)

This project is the second part of a 5-year research program entitled "Malaria Infection Study in Thailand (MIST)" and known as MIST2. MIST2 primary objectives are to assess the safety and

feasibility of blood-stage controlled human *P. vivax* malaria infection (CHMI) in healthy adult Thai volunteers through experimental injection of cryopreserved *P. vivax* infected erythrocytes, and to choose the optimal inoculation dose for future *P. vivax* CHMI studies. In this study, blood-stage CHMI will be conducted in 8 volunteers per inoculum stock who will be infected with *P. vivax* by experimental injection with cryopreserved *P. vivax* infected erythrocytes, which were collected in MIST1. As there are currently 2 stocks of inocula from 2 volunteers in the MIST1 study, which have different quantity and stage of parasites. The total number of volunteers would be up to 16. The volunteers will be monitored closely as in-patients in the Hospital for Tropical Diseases, and will be treated according to the Research Proposal Submission Form.

A10.2 Part of study conducted by PI

☑ Whole Study
☐ Part of the Study

A11. SOURCE(S) OF FUNDING/ SPONSOR(S) AND BUDGET (Information required for review and consideration)

☑ Funded by: Wellcome Trust
Budget amount: 2,000,000 THB
☐ Expecting fund from: ……
Budget amount: ……..…….…….

A12. DECLARE CONFLICT OF INTEREST

The Co-Principal Investigators and the Investigators declare they have no conflict of interest.

PART B: DETAILS OF THE STUDY (Describe only the responsibilities of the PI)

B1. BACKGROUND AND RATIONALE

The *P. vivax* malaria problem

Plasmodium vivax (P. vivax) is one of the five *Plasmodium* species to cause human malaria and accounts for the most cases of non- falciparum malaria worldwide. It is now the most widespread human malarial infection in endemic areas outside Africa. The main reasons for the wide geographical distribution of *P. vivax* are likely to be related to the following aspects of *P. vivax* biology: (1) its ability to **relapse** from dormant liver stage (hypnozoite), (2) its **high transmission potential** due to early production of gametocytes, high infectivity to mosquitoes and shorter development cycle in the vector - host compared to other plasmodia (1, 2).

P. vivax has been considered as causing 'benign' malaria, but recent large case series have demonstrated that *P. vivax* infection is associated with significant morbidity and mortality (3).

Clinical cases are not only due to primary infection following the infected mosquito bite but also relapses from the hypnozoite stage, which occur weeks to years after primary infection (4). Standard schizonticidal regimens are not effective against the hypnozoite. Radical cure of the hypnozoite stage requires therapy with the 8-aminoquinolines primaquine and tafenoquine. However, both medications carry a significant risk of severe haemolytic anemia in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) (5). There is no cheap, reliable point-of-care G6PD test, so many *P. vivax* patients do not currently receive adequate anti-hypnozoite treatment. More recently, the importance of the cytochrome P450 enzyme CYP2D6 in the metabolism of primaquine to the active metabolite has been recognized (6) (7). A common polymorphism in the CYP2D6 gene for the cytochrome P450 enzyme results in poor conversion of primaquine to the active form, resulting in treatment failures. It is estimated that these two factors combined may make nearly 40% of the population at risk of *P. vivax* infection potentially ineligible for primaquine therapy (5).

Recent calls for control and 'eradication' of malaria worldwide have focused attention on this neglected disease and the need for development of an effective *P. vivax* vaccine to be used alongside current control methods. Consequently, the revised Malaria Vaccine Technology Roadmap to 2030 now recognizes the importance of *P. vivax* and calls for development of a vaccine to achieve 75% efficacy over two years – equally weighted with *P. falciparum* in an era of renewed political will to move towards malaria elimination and eradication (8) (9). More recently, research into vivax malaria has increased with candidate vaccines being developed and taken forward to clinical trial (10) (11).

Challenges and directions in *P. vivax* vaccine development.

***P. vivax* malaria vaccine approach**

Most of the *P. vivax* malaria vaccine candidates that are currently under development target individual stages of the *P. vivax* parasite's life cycle. Several highly abundant parasite proteins have been identified as targets of natural immunity and in the recent years the list of possible candidates has expanded.

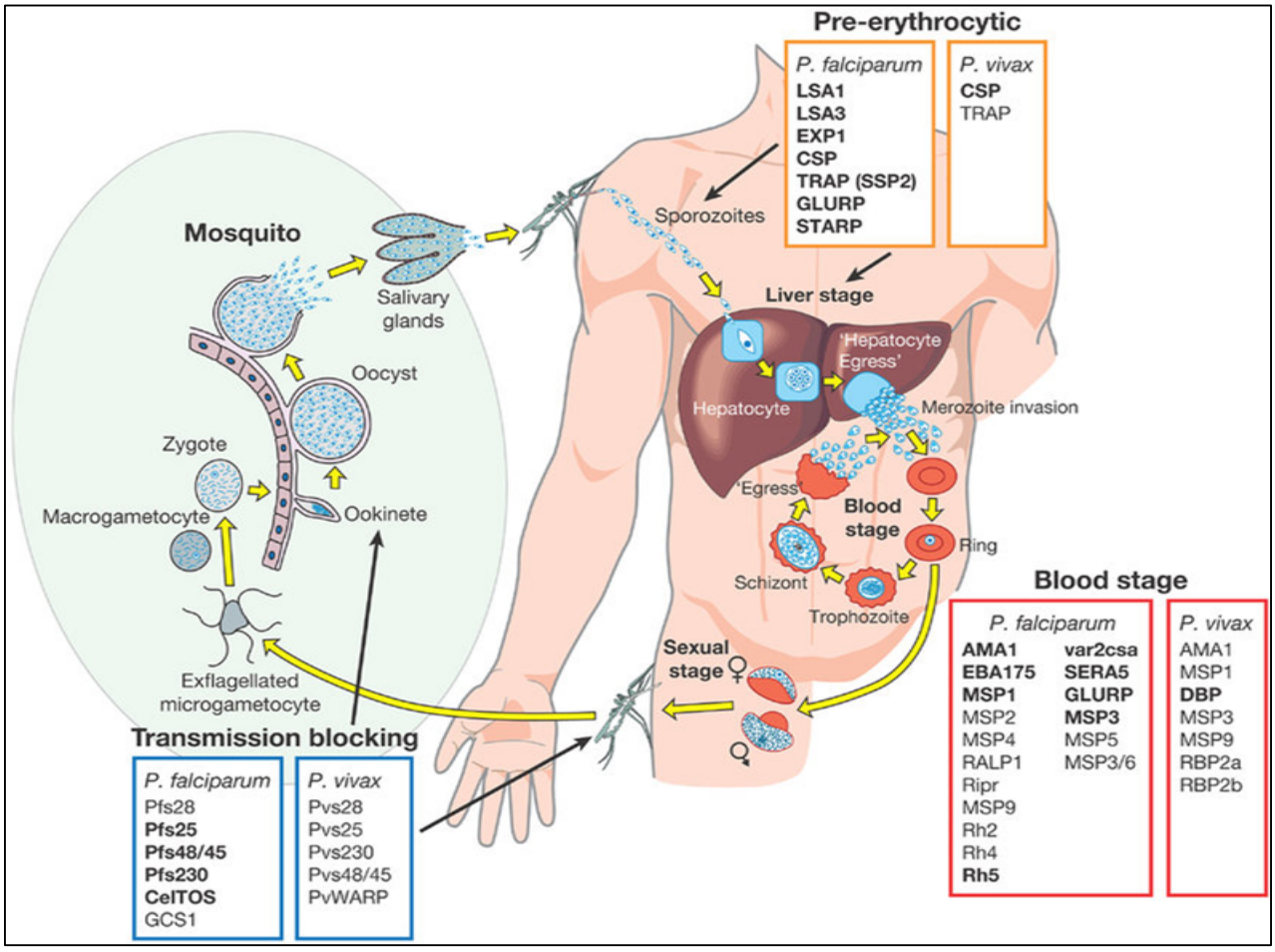
Subunit vaccines for *P. vivax* have been developed based on these candidate antigens. There are three major approaches for the development of malaria vaccines that correspond to the three stages of the parasite's life cycle as follow:

- **Pre-erythocytic antigens;** vaccine using these antigens aim to **prevent initial infection** by targeting the infective stage (sporozoites) that were introduced by the mosquito bite. Alternatively, these vaccines can target antigens expressed by liver stage parasites to prevent the emergence of merozoites into the blood stream e.g. *P. vivax* circumsporozoite protein (PVCSP), *P. vivax* thrombospondin related adhesive protein (PvTRAP)
- **Blood stage antigens**; since all of the symptoms of malaria occur during this stage, the majority of vaccines targeting antigens expressed during the blood stage are designed primarily to **prevent disease or morbidity and mortality associated with the disease** when parasites are in red blood cell (RBC). One approach is to target **merozoite antigens** to

prevent red blood cell invasion and reduce the density and prevalence of parasites in the infected host RBC e.g. receptor-binding region II of *P. vivax* Duffy binding protein (PvDBPII), *P. vivax* merozoite surface proteins (MSP 1), *P. vivax* apical merozoite antigen 1 (PvAMA1), *P. vivax* reticulocyte binding protein/ homolog (PvRBP)

- **Transmission blocking antigens;** the transmission blocking vaccines target antigens expressed during parasite lifecycle stages in the mosquito (e.g. gametocyte or oocyst antigens). Transmission blocking vaccines aim to block malaria transmission from mosquitoes to humans by **preventing the malaria parasite from developing in the mosquito**. Although these vaccines would not directly prevent infection or disease, they would assist elimination efforts through **preventing the onward transmission** of infections e. g. parasite proteins expressed on surface of zygote (Pvs25) and ookinete (Pvs28), alanyl aminopeptidase 1 (AnAPN1), a highly conserved midgut surface antigen of *Anopheles* mosquitoes that is essential for ookinete invasion and development). (Table 1, Figure 1)

Figure 1. Malaria vaccine leading candidate antigens



Ref. Barry and Arnott et al. Frontiers in Immunology, 2014 (359)

Table 1. *P. vivax* malaria vaccine candidates under development

Vaccine candidate	Development Phase	Lifecycle stage	Antigen	Delivery system
VMP001	Phase I/IIa END	Liver-stage	PvCSP	Rec. protein-AS01B
CSV-S,S	Pre-clinical	Liver-stage	PvCSP	HBsAg fusion-AS01B
PvCSP-LSP	Phase I END	Liver-stage	PvCSP	Synthetic peptides-Montanide ISA 720
ChAd63-PvTRAP/MVA-PvTRAP	Pre-clinical	Liver-stage	PvTRAP	Prime-boost, viral vectors
PvDBPII-DEKnull	Pre-clinical	Blood-stage	PvDBP	Rec. protein
PvDBPII	Phase I	Blood-stage	PvDBP	Rec. protein-GLA-SE
PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Rec. protein- Matrix-M1
ChAd63-PvDBPII/MVA-PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Prime boost, viral vectors
PvMSP119	Pre-clinical	Blood-stage	PvMSP1	Rec. protein-Montanide ISA720
Pvs25H	Phase Ia END	Transmission-stage	Pvs25	Rec. protein-Alhydrogel; Rec. protein-Montanide ISA 51
Pvs28	Pre-clinical	Transmission-stage	Pvs28	Rec. protein-adjuvant
Pvs25-IMX313	Pre-clinical	Transmission-stage	Pvs25	Rec. protein-adjuvant
AnAPN1	Pre-clinical	Mosquito midgut Ag	AnAPN1	Rec. protein-adjuvant

Why controlled human challenge trial?

Conventional vaccine development is complex. The average development timeline is between 8 and 18.5 years, estimated costs are $200 million to $900 million, and the probability of success of less than 10% (12, 13). Vaccine clinical trials aim to answer questions related to the immune response, immune correlates of protection, protective efficacy, therapeutic window, and target populations. It is expected that most vaccines will fail because of poor “immune correlates of infection” and “protective efficacy”. Increasing recognition of this critical bottleneck in conventional vaccine

development has led to demand for a proof-of-concept clinical trial: **the controlled human challenge trial**.

In July 2014, the World Health Organization (WHO) held a consultation on Clinical Evaluation of Vaccines: regulatory expectations and controlled human challenge trials were considered important issues for facilitating vaccine development (14). Controlled human challenge trials are thus a prime example of ways in which clinical development of vaccines can be accelerated. Controlled human challenge trials in malaria initially raised ethical debate, but have now gained acceptance through demonstrating their potential and the fact that they have been safely performed in >3,000 volunteers all over the world (15).

The development of a safe and reproducible *P. vivax*-controlled challenge model in humans could greatly accelerate clinical development of *P. vivax* vaccines, through selecting efficacious candidates for further clinical testing in more expensive and logistically challenging studies in the field.

***P. vivax* human challenged study in Thailand**

This study is part of the long term project "Malaria Infection Study in Thailand (MIST)" funded by Wellcome Trust to develop the feasibility of *P. vivax* controlled human challenge trial in an endemic area. The following section provides an overview of the projects in this program, which comprises multiple studies to be conducted conduct over the next 5 years in the Faculty of Tropical Medicine, Mahidol University, Thailand.

Most vivax malaria challenge studies performed so far have been in non-endemic settings such as in the US, UK, and Australia. However, the findings in those settings may not be extrapolatable to the target population for future vaccine deployment, which is more heterogeneous both in terms of vivax immunity and genetic background. The best volunteer population in which to test new vaccines is the eventual target population for vaccine deployment. To date, the MIST 1 is the only CHMI to be performed in a *P. vivax* endemic population in Asia.

P. vivax human challenge studies conducted in Thailand would allow us to understand the immunological correlates of protection in an endemic setting, thereby informing the development of new *P. vivax* vaccine candidates and rapid testing of the protective efficacy of candidate vaccines in the at-risk population in which they will be deployed. Advancing the development of such methods needs renewed emphasis on understanding the biology, pathogenesis and transmission of *P. vivax*.

The overall objectives of the MIST program

- **Proof of feasibility and safety** study and providing *P. vivax* **banked infected blood inocula** for future *P. vivax* blood stage human challenge studies.
- Test the protective efficacy of pre-erythrocytic, blood stage and (ultimately) transmission blocking *P. vivax* **vaccine candidates** in the **target population.**
- Characterise correlates of *P. vivax* immune protection in the **target population**.
- Test the efficacy of **new drug candidates** under development.

- To achieve these major aims written above, the programme will compose of 4 to 7 studies. Each subsequent study will be informed by the previous studies in the series and each study will be covered by separate protocols with separate Ethics Committee submissions and approvals.

- The **MIST2 study (covered by this current protocol)**: Blood stage inoculum human challenge in volunteers from target population (using banked blood from MIST1) **to find the proper dose** for the subsequent studies assessing blood and transmission stage vaccine candidates.

The MIST2 study

During mid- year 2020, the Faculty of Tropical Medicine Ethics Committee (FTM-EC) and the Oxford Tropical Research Ethics Committee (OxTREC) approved the protocol "A clinical study to assess the feasibility of a controlled human *Plasmodium vivax* malaria infection model through experimental sporozoite infection in Thai adults (MIST1)", MAL19002 (FTM-EC #TMEC19-067, OxTREC # 43-19 The study was registered to the Clinicaltrials.gov, # NCT04083508.

The MIST1 study aimed to assess the safety and feasibility of controlled human *P. vivax* malaria infection following infection by the natural route of delivery – mosquito bite. Another major objective of the study was to provide a **source of *P. vivax* infected blood to use in future vaccine efficacy trials of a blood- and transmission-stage vaccines.**

During October- December 2020 sporozoite inoculation by mosquito bite in two healthy subjects was carried out successfully in the Hospital for Tropical Diseases and two blood banks were prepared for the challenge stocks to be used in MIST2 and further blood-stage vaccine trials.

This MIST2 protocol is a blood stage inoculum dose finding study. It is a blood-stage *P. vivax* human challenge study with the primary aim of assessing the safety and feasibility of a challenge model using two banks of cryopreserved *P. vivax* infected erythrocytes for the first time (using the inocula

produced from MIST1) to identify the dose of the inocula to be used in future studies. Four different doses of inoculum will be assessed, by injection at four dilutions of each banked blood, with a view to identifying the lowest concentration producing a reliable infection within a practicable timeframe. This study will serve as the basis for a challenge model for future *P. vivax* candidate vaccine efficacy studies.

Blood stage *P. vivax* vaccine experience

There have been 8 studies of blood-stage *P. vivax* human challenge, of which 5 were published. Five studies were conducted at the Queensland Institute of Medical Research (QIMR), Brisbane, Australia, and 3 studies have been recently conducted by the University of Oxford, UK. The details of the currently unpublished Oxford studies are as follows:

In 2018, Oxford University undertook the first proof-of-concept studies in Europe of *P. vivax* human challenge (ClinicalTrials.gov: NCT03377296). Two healthy UK adult volunteers were safely infected with a clonal strain of *P. vivax* by the bite of infected mosquitoes transported from Thailand. And 250 mL blood was obtained from both volunteers at a prespecified parasitaemia/clinical threshold, leading to the production of a *P. vivax*-parasitized erythrocytes (pRBC) for use in future blood-stage human challenge studies as summarized below.

VAC 069 A-F (NCT03797989) was the first blood-stage human challenge study using pRBCs from a sporozoite inoculation study by mosquito bite. The safety and feasibility of blood-stage infection at 3 different dilutions (whole vial or a neat dose, 1:5 dilution and 1:20 dilation) of thawed inoculum were tested in 6 malaria-naïve healthy UK adult volunteers (2 subjects x1 dilution strength) (VAC069A). Intravenous inoculation of volunteers was completed without complication. All volunteers were diagnosed within 12.5-16.5 days of infection and all had detectable gametocytaemia by qPCR within 1-3 days of peak parasitaemia. After antimalarial treatment with artemether/lumefantrine or atavoquone/proguanil, qPCR was negative by 6 days post-treatment initiation in all volunteers. The full study will be reported soon (Minassian *et al.,* in preparation).

The majority of volunteers (4/6) experienced either no or mild AEs, whilst 2/6 volunteers reported malaria related clinical signs and symptoms. The majority of the AE symptoms resolved within 24 hours after the treatment initiation. Of the laboratory AEs recorded, transient lymphocytopenia and derangement of alanine transaminase (ALT) was seen in 2 volunteers. Both volunteers were asymptomatic.

Based on these infectivity data, and accounting for possible variation in the number of pRBC between thawed cryopreserved vials, an inoculum dilution of 1:10 was selected for future *P. vivax* blood-stage human challenge studies. The inoculum dilution of 1:10 was further tested in 2 malaria-naïve healthy UK adult volunteers (VAC 069 B) and showed qPCR positivity on day 9.

The VAC 069 protocol was also designed to include re-infection (re-challenge blood -stage human control trial) and immunity studies (VAC 069 C - F). All re-challenge blood-stage human challenges were performed in parallel with leading candidate blood-stage vaccine efficacy studies

B2. STUDY OBJECTIVES AND ENDPOINTS

Primary objectives:

1. To assess the safety and feasibility of blood-stage controlled human *P. vivax* malaria infection in healthy adult Thai volunteers through experimental injection of cryopreserved *P. vivax* infected erythrocytes at different doses
2. To choose the optimal inoculation dose for future *P. vivax* CHMI studies.

Primary endpoints:

1. Safety and feasibility of primary *P. vivax* blood-stage CHMI, as measured by (S)AE occurrences and successful infection (development of detectable persistent parasitaemia by thick blood film +/- clinical symptoms)
2. Choosing the optimal inoculation dose for future *P. vivax* CHMI studies, which will be the lowest concentration that produces a reliable infection within a comparable timeframe as compare to the highest concentration.

Secondary objectives:

1. To assess the immune response to *P. vivax* infection in volunteers, through experimental injection of *P. vivax* infected erythrocytes
2. To assess gametocytaemia during *P. vivax* infection in volunteers, through experimental injection of *P. vivax* infected erythrocytes
3. To assess transmission of gametocytes from infected volunteers to *Anopheles* mosquitoes

Secondary endpoints:

1. Immune response to experimental *P. vivax* infection through blood-stage challenge, as measured by antibody, cytokines, B cell and T cell responses
2. Gametocytaemia, as measured by qPCR in experimental *P. vivax* infection through blood-stage challenge
3. Transmissibility of gametocytes from the infected volunteer to *Anopheles* mosquito vector

B3. STUDY SITE(S)

Clinical Therapeutics Unit (FTMCTU), Hospital for Tropical Diseases, Mahidol Vivax Research Unit (MVRU), Medical Entomology Department, Tropical Medicine Diagnostic Reference Laboratory (TMDR), and Malaria Laboratory, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

B4. STUDY TIMELINES:

1. There will be an up to 14 days screening process prior to the malaria challenge day.
2. The duration that individual volunteers will be in the study is 1 year from starting anti-malarial treatment until the end of the follow-up

3. After completion of the last volunteer's last visit, there will be an estimated 3 months for database lock and an estimated 3 months to analyse the data.
4. The total duration of this study is approximately 1 year and 9 months starting after EC approvals.

B5. STUDY POPULATION AND SAMPLE

B5.1 Target population

Sixteen healthy Thai adults, aged from 20 to 55 years with at least an undergraduate degree, will be recruited at the FTMCTU at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. More volunteers would be required if there are more stocks of inoculum in the future.

B5.2 Subject selection criteria

B5.2.1 Subject/inclusion criteria

The volunteer must meet all of the following criteria to be eligible for the study:

1. Healthy Thai adult aged 20 to 55 years with weight at least 50 kg.
2. Red blood cells positive for the Duffy antigen/chemokine receptor (DARC)
3. Women only: Must practice continuous effective contraception for the duration of study period until 3 months post-challenge.
4. COVID-19 vaccination at least two doses of COVID-19 vaccines approved by WHO.
5. Agreement to refrain from blood donation during the course of the study and for 1 year after the initiation of antimalarial treatment.
6. Willing to be admitted in the Hospital for Tropical Diseases for clinical monitoring, until antimalarial treatment is completed and their symptoms are settling.
7. Willing to take a curative antimalarial treatment following CHMI.
8. Willing to reside in Bangkok and its vicinity for 2 months after malarial treatment initiation.
9. Able to read and write in Thai.
10. Provide written informed consent to participate in the trial
11. Answer all questions on the informed consent quiz correctly
12. Educational level: has at least an undergraduate degree

B5.2.2 Subject/exclusion criteria

The volunteer **MUST NOT** enter the study if any of the following apply:

1. Positive malaria qPCR **OR** malaria film
2. Presence of any medical condition (either physical or psychological) which in the judgment of the investigator would place the participant at undue risk or interfere with the results of the study (e.g. serious underlying cardiac, renal, hepatic or neurological disease; severe malnutrition; congenital defects or febrile condition)
3. Presence of chronic disease or chronically use of medication

4. Use of systemic antibiotics with known antimalarial activity in the 30 days before challenge (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin)
5. Use of immunoglobulins or blood products (e.g. blood transfusion) at any time in the 1 year preceding enrolment
6. Receipt of an investigational product, any vaccine in the 30 days preceding enrolment (D0), or planned receipt during the study period
7. Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data or the *P. vivax* parasite as assessed by the Investigator.
8. Any confirmed, or suspected immunosuppressive, or immunodeficient state, including HIV infection, asplenia, history of splenectomy, recurrent, severe infections, and chronic infection
9. Immunosuppressant medication within the past 6 months preceding enrolment (D0) (inhaled and topical steroids are allowed)
10. History of allergic disease or reactions likely to be exacerbated by malaria infection
11. Female participant who is pregnant as evidenced by positive beta-human chorionic gonadotropin (β-HCG) test, lactating, or planning pregnancy during the course of the study
12. Contraindications to the use of antimalarial treatment (e.g. chloroquine, atovaquone / proguanil or dihydroartemisinin/piperaquine)
13. Use of medications known to have a potentially clinically significant interaction with the antimalarial drug that will be used in this study (chloroquine, atovaquone / proguanil or dihydroartemisinin/piperaquine)
14. Known existing positive family history in both 1st AND 2nd degree relatives < 50 years old for cardiac disease
15. History of cardiac arrhythmia, including clinically relevant bradycardia
16. Family history of congenital QT prolongation or sudden death
17. Any clinical condition, including using medications, known to prolong the QT interval.
18. Screening electrocardiogram (ECG) demonstrates a QTc interval ≥ 450 ms.
19. Suspected or known or history of alcohol abuse
20. Suspected or known or history of drug abuse.
21. Concurrently participating in another clinical study, at any time during the study period
22. Haemoglobin < 11 g/dL
23. Positive hepatitis B surface antigen or seropositive for hepatitis C virus
24. Finding on safety laboratory values as defined below:

- Abnormal AST (AST > 40 U/L for male, and > 32 U/L for female [upper normal range])
- Abnormal ALT (ALT > 41 U/L for male, and > 33 U/L for female [upper normal range])
- Abnormal serum creatinine (Scr) (Creatinine [Cr] > 1.17 mg/dL for male, and > 0.95 mg/dL for female [upper normal range])
- Abnormalities corrected calcium and magnesium blood levels

25. Blood group Rhesus negative
26. Blood incompatibility to the inoculum
27. Positive for COVID-19 diagnosed by RT-PCR

B5.3 Sample size calculation

In order to find the optimum dose of each blood-stage inoculum (the lowest concentration that the volunteers reliably develop infection, i.e., 2/2, within the comparable time frame as compare to the highest concentration and within 21 days), one blood-stage inoculum stock will be tested in 4 different doses: whole dose inoculum (neat vial), 1:5, 1:10, and 1:20 dilution. Each dose will be inoculated into two volunteers resulting in 8 volunteers. As two stocks of blood-stage inoculum were prepared from MIST1, 16 volunteers will be recruited: 8 to be challenged with inoculum stock 1, and 8 with inoculum stock 2.

B5.4 Recruitment Methods

Volunteers will be screened and recruited at the FTMCTU, Faculty of Tropical Medicine, Mahidol University in Bangkok following Recruitment Process for Healthy Volunteer Standard Operating Procedure (SOP).

In brief, the volunteers may be recruited by use of an advertisement approved by the ethics committee and distributed or posted in the following places:

- On a MIST website operated by the study group
- In public places with the agreement of the owner / proprietor
- Via presentations (e.g. presentations at lectures or invited seminars)

In addition, we may contact individuals who have participated in previous clinical trials from the FTMCTU's databases. These volunteers will have expressed an interest in receiving information about all future studies in FTMCTU for which they may be eligible.

B5.5 Withdrawal/discontinuation criteria

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2013) and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. In addition, the volunteer may withdraw/be withdrawn from further study procedures at any time in the interests of the volunteer's health and well-being, or for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Volunteer non-compliance with study requirements.
- An AE which requires discontinuation of the study involvement or which results in inability to continue to comply with study procedures.
- Current COVID-19 infection during a participant's admission in the hospital

The medical monitors may recommend withdrawal of volunteers. The reason for withdrawal from further study procedures will be recorded in the Case Record Form (CRF). If a volunteer withdraws

after having completed a course of antimalarials, as much long-term safety data collection as possible, including procedures, such as safety bloods, will continue to be collected, with the agreement of the volunteer. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilized, or a non-trial related causality has been assigned.

If a volunteer withdraws from the study after challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of antimalarial therapy must be completed with standard chloroquine treatment. The importance of this will be emphasized to volunteers at screening. If a volunteer refuses to take antimalarial therapy after malaria diagnosis, a rapid assessment of mental state and capacity will be undertaken, with the involvement of psychiatric and infectious diseases specialists. If necessary and if in accordance with the law in Thailand, the volunteer may be detained for appropriate medical management.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Similarly, all data collected up to the point of withdrawal will be stored, unless they specifically request for it to be destroyed. Volunteers are free to request that their blood samples be destroyed anytime during or after the study.

In all cases of volunteer withdrawal, except those of complete consent withdrawal, safety data collection will continue if volunteers have already undergone challenge process.

B5.6 Multi-site research

- Not applicable

B6. RESEARCH METHODOLOGY

B6.1 Details of study design

Summary of trial design

Controlled *Plasmodium vivax* human malaria infection through experimental inoculation of cryopreserved infected erythrocytes in healthy Thai adults, with a randomized double-blind design.

Overview

This blood-stage *P. vivax* human challenge study conducted for the first time in Asia has two primary aims: (1) to assess the safety and feasibility of the challenge model using the bank of cryopreserved *P. vivax* infected erythrocyte inocula prepared during the MIST1 study, and (2) to determine the optimal inoculation dose for future *P. vivax* CHMI studies. This will be assessed by intravenously injecting the volunteers with four different doses of inoculum: whole dose and 1:5, 1:10, and 1:20 dilutions. If safe and feasible, this study will serve as the basis for a challenge model for future *P. vivax* candidate vaccine efficacy studies. The secondary objectives are to determine the

immune response to *P. vivax* infection, the resulting gametocytaemia, and transmissibility of the gametocytes from the infected volunteers.

Healthy, Thai adults aged between 20 and 55 years will be recruited and randomized at the Faculty of Tropical Medicine, Mahidol University in Bangkok.

CHMI will be induced by injection of *P. vivax* infected erythrocytes and all follow-up in the post-challenge period will be performed at the Faculty of Tropical Medicine, Mahidol University in Bangkok.

Volunteers will have blood taken at regular intervals post-CHMI to assess the immune response to *P. vivax* infection, as well as parasite growth dynamics and gametocytaemia. We will also assess transmission of *P. vivax* gametocytes from the infected volunteers to mosquitos using a membrane feeding assay (MFA).

Close monitoring will continue until volunteers meet criteria for treatment or until 21 days after challenge, when treatment will be started empirically.

Therapy will be with a standard course of chloroquine where not contraindicated. As infection will be induced via intravenous injection of blood-stage parasites, there will be no liver-stage infection and no hypnozoite formation, thereby eliminating the need for radical cure with primaquine therapy. Follow-up at study site will be up to 1 year after antimalarial treatment initiated.

B6.2 Details of procedures for specimen/data collection

B6.2.1. Screening and eligibility assessment

B6.2.1.1. Screening visit

The screening will aim to recruit 16 healthy volunteers.

Screening visits may take place up to 14 days prior to enrolment. Informed consent will be conducted at screening. If consent is given, the screening procedures will be undertaken, including testing for Duffy antigen/chemokine receptor (DARC) positivity, blood diseases, blood biochemistry, blood-borne infections, and serum pregnancy test. The full list of laboratory tests and the schedules of procedures is listed in Table 4.

Abnormal clinical findings from the medial history, physical examination, or laboratory tests at any point in the study will be assessed according to the scales in APPENDIX C: Safety terms and administrative information. If a test is deemed clinically significant it may be repeated to ensure it is not a single or spurious occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer.

Cross matching will be performed to check blood compatibility between volunteer and the blood inoculum.

Exclusion of the volunteer from enrolling in the trial or withdrawal of a volunteer from the trial will be at the discretion of the Investigators.

B6.2.1.2. Day before CHMI (Admission day; D -1)

Any new medical issues or symptoms that have arisen will be assessed. All eligible volunteers will have physical examination/observations will be performed during this visit. Serum β-HCG (woman), malaria diagnosis, complete blood count (CBC), biochemistry, and COVID-19 testing (RT-PCR will be tested during this visit. In addition, COVID-19 serology test will also be done to look at participants' immunity from the current/previous COVID-19 infection (asymptomatic) and/or prior immunization. G6PD test and malaria immunological profiles will be tested for baseline information only and will not be used as exclusion criteria. CMV IgM/IgG antibody, EBV VCA IgM/IgG antibody, and anti EBNA and EA complex antibody will be tested. Results of blood taken at this visit must be available and reviewed prior to challenge.

B6.2.1.3. Day of CHMI (D0)

Half of the number of challenged volunteers must also be available during the period of the malaria challenge (during the whole inoculation process) for back-up, in case one of the volunteers in the batch withdraws at the last minute or any unexpected event occurs. This means that confirmation of whether or not a back-up volunteer will be needed for the challenge will not be made until the challenge (the whole inoculation process) has been successfully completed.

CHMI will be administered according to local Standard Operating Procedure (SOP). This will include:

- Interim history and examination of the injection site and any body systems felt to be necessary by the Investigator and verification of eligibility / contraindications.
- Physical observations (including respiratory rate, pulse rate, blood pressure and temperature), with recordings to be repeated if this is longer than 60 minutes before administration of the parasitized erythrocyte inoculum.
- Intravenous access via cannulation in a forearm vein, flushed with normal saline.
- For each volunteer, the inoculum must be injected within 4 hours of the inoculum being thawed, followed by a further normal saline flush.
- Physical observations will be repeated at 15 minutes post-administration of inoculum and again at 1 hour, in order to assess for immediate adverse reactions, with regular visual observation throughout, in line with The British Society for Haematology Guideline for the administration of blood products (23). If the volunteer shows signs or symptoms of a transfusion reaction, observations will be repeated and the volunteer will be medically reviewed by a physician.
- Volunteers will be observed for at least 60 minutes; however, this period may be extended if there are any clinical concerns.

- If there have been no symptoms or signs indicative of a transfusion reaction, the cannula will be removed after 1 hour.

B6.2.2. Days 1-28 post CHMI (D1 to D28)

This section describes the clinical procedures for evaluating study volunteers and management **after** challenge.

B6.2.2.1 Day 1 post challenge to malaria qPCR show positive ($D_{qPCR}+$)

The volunteers will be assessed once daily from day 1 post challenge until malaria qPCR becomes positive ($D_{qPCR}+$). The assessment includes a clinical well-being check, and the volunteers will be questioned whether they have experienced any malaria-related symptoms (such as fever, chills, sweating, malaise). The once daily general vital signs and physical examination will be done. Blood will be drawn once daily for monitoring blood parasitaemia (malaria blood film, qPCR, and gametocyte qPCR) and membrane feeding to assess the transmissibility of the gametocyte. Immunological profile and CBC will be performed on day 4 and D_{qPCR+}. If volunteer present with a clinical feature(s) of COVID-19 at the discretion of the trial physician, and the malaria blood film and malaria qPCR are both negative, COVID-19 testing will be performed. The complete list of laboratory procedures is shown in Table 5.

B6.2.2.2 Day after qPCR+ (Day $_{After\ qPCR+}$) *to day of reach treatment criteria ($D_{reach\ treatment\ criteria}$)*

The clinical well-being will be checked. Blood will be drawn twice daily for monitoring blood parasitemia (malaria blood film, qPCR, and gametocyte qPCR) and membrane feeding to assess the transmissibility of the gametocyte. Malaria immunology, CBC, and blood biochemistry will be assessed on $D_{reach\ treatment\ criteria}$. In addition, blood collection will be performed to test for CMV IgM/IgG antibody, EBV VCA IgM/IgG antibody, and anti EBNA and EA complex antibody at 2-4 weeks after the inoculation.

Volunteers will be assessed after challenge according to the schedule in Table 5. Physical observations will be performed. Venepuncture will be performed as per schedule of attendance (Tables 5-6).

Volunteers will be questioned as to whether they have:
- o Experienced any of the foreseeable symptoms of malaria.
- o Experienced any other symptoms.
- o Taken any medications including over the counter medications.

Full physical examination will be performed if deemed necessary by the Investigators. The severity of symptoms will be assessed using grading criteria summarised in APPENDIX C: Safety terms and administrative information.

Antimalarial treatment will be instituted according to criteria described below (Table 2).

If the antimalarial treatment criteria have not yet been met, the treatment shall be initiated at day 21 after challenge (D21). Subjects will be closely monitored until completion of antimalarial therapy, as described above. Summary of clinical assessment and laboratory including safety blood tests is shown in Table 5 and detail of blood volume and blood collection tube is shown in Table 6.

B6.2.2.3 Antimalarial treatment

A study physician will then immediately prescribe antimalarial treatment with chloroquine, in accordance with Table 3. Any side effect from the antimalarials will be rapidly dealt with in a controlled environment. They will continue to have blood films and qPCR once daily until clinically recovered AND two consecutive malaria blood films are negative (completion of the chloroquine treatment course). The volunteers will be then discharged from the hospital. Before discharge from the hospital, COVID-19 testing (RT-PCR) and COVID-19 serology test will be carried out to confirm that subjects did not acquire COVID-19 during their inpatient stay.

If any volunteer reaches day 21 post-challenge without a positive malaria blood film, they shall be started on a 3-day course of antimalarial (chloroquine).

If any contraindications to chloroquine are identified, an alternative antimalarial dihydroartemisinin + piperaquine or Malarone (atovaquone + proguanil) will be prescribed. If a volunteer is unable to tolerate an oral antimalarial, the appropriate parenteral antimalarial therapy will be prescribed.

If a volunteer withdraws/is withdrawn from the study after challenge but before reaching the criteria for malaria treatment, then a **complete, appropriate, curative course of antimalarial therapy must be completed.** The importance of this will be emphasized to volunteers at screening.

Table 2*:* Antimalarial treatment criteria.

Malaria treatment Start Criteria	• Symptoms suggestive of malaria infection with the presence of blood parasitaemia by microscopy. • Asymptomatic with ≥ 50,000 parasites/mL by microscopy OR • Asymptomatic and no parasitaemia until Day 21 Note: In case the volunteers develop symptoms suggestive of malaria infection, but no parasitaemia, other causes of symptoms should be considered and treated as appropriate.

Table 3: Chloroquine treatment dose*

Weight	Day 1	Day 2	Day 3
	CQ Tab	CQ Tab	CQ Tab
50 kg or more	4	4	2

* National Malaria Treatment Guideline 2019 (Thailand), CQ 150 mg base/tablet

Supportive treatments and medications

Any malaria symptoms (e.g. fever, nausea, vomiting) will be relieved accordingly. Paracetamol (500 mg orally up to four times a day) and dimenhydrinate (50 mg orally) will be prescribed according to clinical need. Intravenous fluid supplementation may be given following physician decision.

All medications used in the trial will be recorded in the medical record.

If the volunteers meet the criteria for severe malaria (by WHO definition) or fail to improve within 48 hours of starting anti-malarial therapy, malaria experts in the Faculty of Tropical Medicine will be consulted. The volunteers will be managed according to National Malaria Treatment Guideline (Thailand) as well as the Hospital for Tropical Diseases' standing order/ procedures/ guideline for severe malaria.

The Investigators are able to treat any volunteer for malaria regardless of the blood film microscopy or qPCR result if they are clinically concerned or a volunteer wishes to withdraw from the study.

B6.2.3. Follow up assessment phase (after completion of antimalarial treatment)

On the antimalarial treatment end date, the volunteers will receive full counselling on the signs and symptoms that they have to be able to observe early and report in the diary card and seek medical advice. A diary card to self-document any abnormal symptoms will be given to them, and the contact channel with the study team will again be emphasized. After discharge from the hospital, the follow-up assessment will be performed at FTMCTU as an out-patient on day 7 post treatment initiated (D_{Rx7}), day 28 (D_{Rx28}), day 60 (D_{Rx60}), day 90 (D_{Rx90}), day 180 (D_{Rx180}), and day 1 year ($D_{Rx1yr.}$) post treatment initiation. On the follow-up days, diary cards will be reviewed and collected, a history taken using the clinical well-being checklist, a physical examination will be performed, and AE(s) assessed. Venipuncture will also be performed to detect malaria parasites by blood film and qPCR, and for malaria gametocyte qPCR, MFA, malaria immune response, CBC, and biochemistry according to Tables 5-6. Other study activities will be undertaken according to Table 5.

During this follow up phase, the volunteers will be contacted fortnightly (biweekly) until D_{Rx90} by email / phone call / other social communications e.g. message (SMS), WhatsApp, Line, to ensure they remain well and asymptomatic. The volunteer will have to understand that they must stay in Bangkok until D_{Rx60} and always immediately contact the study team as soon as they observe any abnormal symptoms or would like to contact the team for any other reason.

Table 4: Schedule of clinical and study procedures for volunteers pre-challenge

Event	Screening (within 14 days prior to Day 0)	Screening 2/Admission (Day -1)
Inclusion / Exclusion criteria	X	X
Informed Consent Questionnaire	X	
Informed consent	X	
Medical History	X	X
Physical Examination	X	X
Body weight and height[a]	X	X
Urinalysis	X	
Electrocardiogram	X	
Vital signs[b]	X	X
Blood typing ABO, Rhesus, Crossmatching	X	
Duffy antigen for chemokine receptor (DARC)	X	
CBC	X	X
Haemoglobinopathies and thalassaemia traits*	X	
G6PD*		X
Blood biochemistry[c]	X	X
FBS	X	
HIV, HBV, HCV[d]	X	
Malaria diagnosis (Malaria antigen – blood film and qPCR)	X	X
Malaria exposure*	X	
Malaria immunology*	X	X
β-HCG testing (women only)	X (Serum pregnancy test)	X (Serum pregnancy test)
COVID-19 testing (RT-PCR)		X
COVID-19 serology test		X
CMV and EBV serology		X

Remark:

[a] Height will be measured only at screening visit.

[b] Vital signs include blood pressure, pulse rate, respiratory rate and temperature.

[c] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate, calcium and magnesium), blood urea nitrogen (BUN), creatinine (Cr), eGFR, liver function tests (LFTs)

[d] HIV-I and HIV-II antigen; anti-HCV antibody will be tested. HBV test includes HBsAg, anti-HBc, and anti-HBs.

Table 5: Schedule of Clinic Attendances Post-challenge

Event	**Admission Phase**				**Follow-up Phase**					
Timeline	D0	D1 – D_{qPCR+}	$D_{after\ qPCR+}$ – $D_{reach\ treatment\ criteria}$	$D_{after\ start\ CQ}$ - $D_{d/c}$	D_{Rx7}	D_{Rx28}	D_{Rx60}	D_{Rx90}	D_{Rx180}	$D_{Rx1yr.}$
Window (days)	0	0	0	0	±2	±4	±7	±7	±14	±14
Inclusion/exclusion criteria	X									
Medical history (well-being check list)	X	X (once daily)	X (Twice daily)	X (once daily)	X	X	X	X	X	X
Body weight	X		X ($D_{reach\ treatment\ criteria}$)		X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test (women only)			X (before treatment)							
Vital signs[a]	X	X	X	X	X	X	X	X	X	X
Local & systemic events (AE/SAE)[b]	X	X	X	X	X	X	X	X	X	X
Blood stage challenge	X									
Treatment for Malaria[c]			X (D reach treatment)	X						
Diary card				$D_{d/c}$	X	X	X	X		
Malaria Blood Film		X (daily)	X (twice daily)	X (daily)	X	X	X	X	X	X
Malaria qPCR and Gametocyte qPCR		X (daily)	X (twice daily)	X (daily)	X	X	X	X	X	X
Haematology (CBC)		D4, D_{qPCR+}	X ($D_{reach\ treatment\ criteria}$)		X	X	X	X	X	X
Biochemistry[d]		D_{qPCR+}	X $D_{reach\ treatment\ criteria}$)	$D_{d/c}$	X	X	X	X		X
Malaria Immunology		D4, D_{qPCR+}	X ($D_{reach\ treatment\ criteria}$)		X	X	X	X	X	X
MFA		X (daily)	X (twice daily)	X (daily)	X	X	X	X	X	X
COVID-19 testing (RT-PCR)		X[e]		X (d/c)						
COVID-19 testing (serology test)			X[f]							
EBV and CMV serology			X[g]							

Remark:

[a] Vital signs include blood pressure, pulse rate, respiratory rate and body temperature.

[b] AEs/SAEs will be collected until 1 year after antimalarial initiation.

[c] This treatment period could occur on any day between D6 and D21. Blood draws will continue as per this Table for the relevant study day, up until the time-point of treatment. The treatment will be done on D21 if no criteria meet.

[d] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs)

e In case volunteers have fever but malaria blood film and malaria qPCR are negative, COVID-19 will be tested.

f COVID-19 serology test will be done on the day of discharge.

g EBV and CMV serology will be done at 2-4 weeks after the inoculation. EBV and CMV serology including EBV VCA (IgM)/IgG antibody, anti EBNA and EA complex antibody, and CMV IgM/IgG antibody

Table 6: Blood collection during study period

Phase	Clot blood		EDTA blood		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume (mL)	Test	Volume (mL)	Test	Volume (mL)	Test		
Screening 1	6	Blood typing, Cross matching	24	Malaria antigen (blood film, qPCR)	2	Fasting blood sugar	32	32
		Blood biochemistry						
		HBs profile, anti-HCV		Duffy blood group				
		Serum β-HCG		CBC + Thalassemia				
				Hemoglobinophathies				
				Malaria exposure				
				Malaria immunology				
				HIV-I, HIV-II				
Screening 2 (D-1)	10	Serum β-HCG	16	CBC + G6PD screening test (FST)			26	58
		Blood biochemistry		Malaria immunology				
		CMV, EBV serology		Malaria antigen (blood film, qPCR)				
		COVID-19 serology						
Admission D1 - $D_{qPCR}+$ (~D8)	5	Blood biochemistry ($D_{qPCR}+$)	60	Malaria antigen (blood film, qPCR, qGAM) (once daily)			65	123
				Membrane feeding (once daily)				
				Malaria immunology (D4, $D_{qPCR}+$),				
				CBC (D4, $D_{qPCR}+$)				

Phase	Clot blood		EDTA blood		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume (mL)	Test	Volume (mL)	Test	Volume (mL)	Test		
After $D_{qPCR}+$ to $D_{reach\ treatment\ criteria}$ (D9~D16)	9	Blood biochemistry ($D_{reach\ treatment\ criteria}$) CMV, EBV serology	78 (48+16+12+2)	Malaria antigen (blood film, qPCR, qGAM) (twice daily) Membrane feeding (twice daily) Malaria immunology ($D_{reach\ treatment\ criteria}$) CBC ($D_{reach\ treatment\ criteria}$)			87	210
$D_{After\ start\ CQ}$ - $D_{d/c}$	6	Blood biochemistry ($D_{d/c}$) COVID-19 serology (Dd/c)	16	Malaria antigen (blood film, qPCR, qGAM) (once daily) Membrane feeding (once daily)			22	232
D_{Rx7}	5	Blood biochemistry	18	Malaria antigen (blood film, qPCR, qGAM) Membrane feeding Malaria immunology CBC			23	255
D_{Rx28}	5	Blood biochemistry	18	Malaria antigen (blood film, qPCR, qGAM) Membrane feeding			23	278

Phase	Clot blood		EDTA blood		NaF		Total blood (mL)	Accumulate blood volume (mL)
	Volume (mL)	Test	Volume (mL)	Test	Volume (mL)	Test		
				Malaria immunology				
				CBC				
D_{Rx60}	5	Blood biochemistry	18	Malaria antigen (blood film, qPCR, qGAM)			23	301
				Membrane feeding				
				Malaria immunology				
				CBC				
D_{Rx90}	5	Blood biochemistry	18	Malaria antigen (blood film, qPCR, qGAM)			23	324
				Membrane feeding				
				Malaria immunology				
				CBC				
D_{Rx180}			18	Malaria antigen (blood film, qPCR, qGAM)			18	342
				Membrane feeding				
				Malaria immunology				
				CBC				
$D_{Rx\ 1\ yr.}$	5	Blood biochemistry	18	Malaria antigen (blood film, qPCR, qGAM)			23	365
				Membrane feeding				
				Malaria immunology				
				CBC				

B6.2.4. Details of study clinical procedures/laboratory tests

Procedures will be performed at the time points indicated in the schedule of procedures (Tables 4 - 6). Additional procedures or laboratory tests may be performed, at the discretion of the investigators if clinically necessary. Study procedures will be monitored by the MORU Clinical Trials Support Group.

B6.2.4.1. Clinical procedures

B6.2.4.1.1. Body Weight and height

Body Weight and/or height will be measured at the time points indicated in the schedule of procedures (Tables 4 & 5).

B6.2.4.1.2. Vital signs

Pulse rate (PR), blood pressure (BP), respiratory rate (RR) and body temperature (BT) will be measured at the time points indicated in the schedule of procedures (Tables 4 & 5).

B6.2.4.1.3. Urinalysis

Urine will be tested for the presence of clinically significant proteinuria, glucosuria or haematuria at the screening visit.

B6.2.4.1.4. Electrocardiogram

An electrocardiogram will be performed at the screening visit.

B6.2.4.2. Laboratory procedures

Tropical Medicine Diagnostic Reference Laboratory (TMDR) will be the central reception point for all blood samples collected from FTMCTU, and will follow a sample management system designed by the MORU Sample Management Centre. TMDR will aliquot diagnostic and research samples and be responsible for transfer to designated laboratories for testing and retrieve the result generated from the following laboratories. “In case any laboratories could not perform the tests specified below or stop providing the service, then the tests can be done at other validated laboratory.”

- **Diagnostic Laboratory Unit, Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University**

1. Haematology: complete blood count (CBC) and blood grouping (ABO, Rhesus)
2. G6PD fluorescence spot test
3. Biochemistry: electrolytes, blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs), fasting blood sugar (FBS)
4. Serum beta-human chorionic gonadotrophin (serum β-HCG)
5. Diagnostic serology: HBs profile (HBsAg, anti-HBc, anti-HBs), anti-HCV
6. Crossmatching
7. Urinalysis
8. Urine pregnancy test
9. CMV, EBV serology

- **Tropical Medicine Diagnostic Reference Laboratory (TMDR)/ Microbiology and Immunology Department, Faculty of Tropical Medicine, Mahidol University**
  1. COVID-19 serology (or other validated laboratories)
  2. COVID-19 PCR

- **Malaria Laboratory, Faculty of Tropical Medicine, Mahidol University**
  1. Malaria microscopic examination

- **Molecular Tropical Medicine & Genetics Department, Faculty of Tropical Medicine, Mahidol University**
  1. High volume qPCR for Malaria

- **ATGenes**
  1. Duffy antigen
  2. Haematological diseases*: haemoglobinopathy analysis, CBC, haemoglobin electrophoresis and molecular studies for alpha and beta thalassaemia trait

- **Siriraj Hospital, Mahidol University**
  1. qPCR for HIV-I, HIV-II

- **Mahidol Vivax Research Unit (MVRU), Faculty of Tropical Medicine, Mahidol University**
  1. Background exposure to malaria*: Multiplex Bead Based Immunoassay will be performed to determine the magnitude of antibodies against recent exposure of 5-8 blood-stage *P. vivax* antigens
  2. Malaria and gametocyte qPCR
  3. Immunology: Immunological responses to *P. vivax* infection.
  4. Membrane feeding assay

*This test will be done for data interpretation purpose. It will not be used as the inclusion/exclusion criteria.

Samples may also be sent to collaborating laboratories within and outside Thailand for immunomonitoring and/or harmonisation of key immunological assays. There are two collaborating laboratories:

(1) Ehime University, Matsuyama, Japan, and (2) Kenya Medical Research Institute (KEMRI), Kenya

The AlphaScreen assay will be used to quantify malaria antigens-specific antibodies (up to 300 *P. falciparum* antigens and 300 *P. vivax* antigens). A seropositive cut-off will be set as half the lowest non-negative value of the assayed samples. Positive malaria history refers to a volunteer who previously had a malaria infection leading to antibodies in their serum which reactive to more than 10% of the malaria antigens in the library. This may include human DNA and RNA analysis.

B6.2.5. ***Challenge*** **infection performed in the trial participants**

B6.2.5.1. Description and administration of the **study agents (which is the cryopreserved P. *vivax* infected erythrocytes)**

In this study, the study agent that we apply / administer to the volunteer is not a drug (since it is not a drug trial). It is "cryopreserved P. vivax infected erythrocytes". The total volume of the diluted cryopreserved parasite that will be injected into each volunteer is 5 mL.

B6.2.5.1.1. Source and preparation of the cryopreserved inoculum

The cryopreserved *P. vivax* parasitized red blood cells that will be used were produced *by investigator team* in *December* 20*20*, as part of "A clinical study to assess the feasibility of a controlled human *Plasmodium vivax* malaria infection model through experimental sporozoite infection in Thai adults" ('MIST 1 study', certificate number 2020-038-01, approved by the FTM-EC #TMEC19-067; OxTREC Ref 43-19. The information is as followed.

Cryopreservation and storage of blood bank

P. vivax infected blood processing was conducted within fumigated microbiological safety cabinets. First, the red cells were separated from plasma by centrifugation of the leukodepleted blood before mixing with GMP grade Glycerolyte 57 (at 1:2 erythrocyte to Glycerolyte 57 volume ratio). The first 20% of Glycerolyte 57 was added dropwise with gentle agitation, the suspension was then incubated for 5 minutes at room temperature before the remaining Glycerolyte 57 was added. The RBC-Glycerolyte mixture was aliquoted into 1.5 mL cryovials and frozen at -80^{o}C for one night before transferred to a dedicated liquid nitrogen tank. Confirmation of parasitic density within the blood collected for cryopreservation was performed via microscopy and quantitative PCR on the leukodepleted packed blood samples. Thick film microscopy demonstrated 6 asexual parasites per 1 µL leukodepleted packed blood for Donor 1, and 13 asexual parasites per 1 µL leukodepleted packed blood for Donor 2. Quantitative PCR confirmed the presence of 155,400 genome copies/mL in Donor 1, and 988,600 genome copies/mL in Donor 2.

Sterility and screening of blood-borne and vector-borne infections of cryopreserved blood bank

Sterility and screening for blood-borne and vector-borne infections of cryopreserved blood bank were performed to ensure the safety of cryopreserved blood bank. Real time PCR for HIV-1, HIV-2, HBV, HCV, Dengue, chikungunya, zika, Japanese encephalitis, CMV and EBV, serology for HTLV-1, HTLV-2 and syphilis (TPHA), serology for filaria, and ELISA for mycoplasma were tested from plasma derived directly from blood bank. Blood collected for cryopreservation was sent to FTM hospital for testing the bacterial contamination using hemoculture technique. All tested results were negative for both donors.

B6.2.5.1.2. Preparation of the inoculum

Thawing and washing of the inoculum will be done with commercial solutions for human use and with disposable syringes and needles according to local standard operating procedure. The processing

of the inoculum will be carried out in MVRU's dedicated clean room biosafety level II laboratory at the Hospital for Tropical Diseases, Faculty of Tropical medicine. Sample manipulations will be performed within a safety cabinet that has been fumigated, sterilised and dedicated for this purpose. Deglycerolizing will be performed by sequentially adding 0.1 volume of 12%, 10 volume of 1.6% and 10 volume of 0.9% saline solution. Either one whole vial, containing approximately 0.5 mL of red blood cells, one fifth of a vial, one tenth of the vial or one twentieth of a vial will be reconstituted in 0.9% saline, to a total volume of 5 mL. Five milliliters of each of the diluted inoculum will be drawn in 5 mL syringe and close with sterile cap. The inoculum will be kept in the warm transport box until inoculate to the volunteers. The inoculum must be injected within 4 hours of the inoculum being thawed.

B6.2.5.1.3. Administering the study agent to the volunteers:
The inoculation will take place at the Hospital for Tropical Diseases. The inoculum will be administered by injection into an indwelling intravenous cannula.

The challenge steps:
The volunteer(s) will be hospitalized a night before the inoculation day (D0) to ensure their safety / eligibility and to avoid the traffic burden.

Inoculum will be transported directly from the MVRU clean room lab at 9th floor in a warm box and immediately transferred to a secure room within the Hospital for Tropical Diseases.

The number of volunteers in each group depends on the availability of cryopreserved inoculum stocks. Recently, there are 2 inoculum stocks, so volunteers in group 1 and 2 will be inoculated by inoculum stock#1 and stock#2, respectively. Two volunteers will be challenged with blood-stage inoculum per dose as shown in Table 7. All groups will receive an inoculum of parasitised red blood cells reconstituted in 0.9% normal saline, to a total volume of 5 mL, containing different dilution doses as specified below and as shown in the Table 7.

Table 7 MIST 2 intervention details
Dose finding for 2 cryopreserved inocula in Thai volunteers

Participants per group	Group 1 for inoculum#1	Group 2 for inoculum#2	Intervention
8 volunteers	2	2	Challenge with whole dose blood-stage inoculum (neat)
	2	2	Challenge with 1:5 dilution blood-stage inoculum
	2	2	Challenge with 1:10 dilution blood-stage inoculum
	2	2	Challenge with 1:20 dilution blood-stage inoculum

Participant who withdraws or be withdrawn before inoculation completed will be replaced.

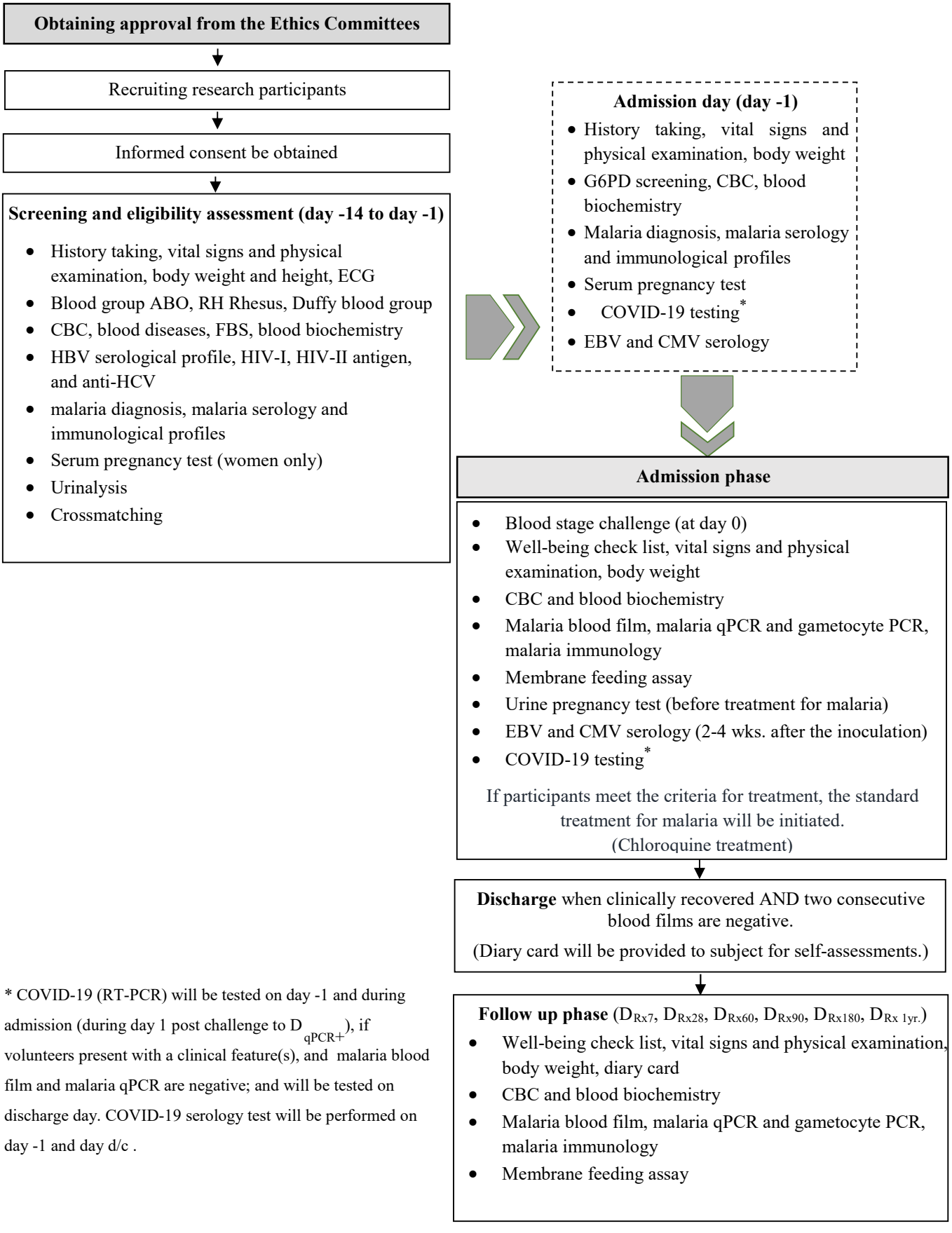
Whole dose: one whole vial, containing approximately 0.5 mL of red blood cells, will be reconstituted in 0.9% saline, to a total volume of 5 mL
1:5 dilution: one fifth of a vial will be reconstituted in 0.9% saline, to a total volume of 5 mL.
1:10 dilution: one tenth of a vial will be reconstituted in 0.9% saline, to a total volume of 5 mL.
1:20 dilution: one twentieth of a vial will be reconstituted in 0.9% saline, to a total volume of 5 mL.

The reconstituted inoculum will be injected via an indwelling cannula, followed by a saline flush.

One volunteer from each group (Phase A and B - Groups 1-4), receiving one of the four concentrations, will be administered the inoculum first. The inoculum will subsequently be administered to each of the second 4 volunteers from each group. This is in case the time between administration of the inoculum affects the viability of the parasites.

Prior to challenge, all volunteers are briefed. The arms will be cleaned by using 70% alcohol according to the Inoculum Administration Work Instruction (WI).

B6.3 Schematic diagram of study design, procedures and stages, step-by-step



* COVID-19 (RT-PCR) will be tested on day -1 and during admission (during day 1 post challenge to D_{qPCR+}), if volunteers present with a clinical feature(s), and malaria blood film and malaria qPCR are negative; and will be tested on discharge day. COVID-19 serology test will be performed on day -1 and day d/c .

B6.4 Specimen and data management

Mahidol Vivax Research Unit (MVRU) will be the central reception point for all blood samples collected from FTMCTU, and will follow a sample management system designed by the MORU Sample Management Centre.

The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH Good Clinical Practice (ICH GCP) E6 (R2) and regulatory and institutional requirements for safety and protection of confidentiality of volunteers. The Co-Principal Investigators are responsible for data management and for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The study staff will enter the data into the volunteers' CRFs, which will be in a paper and/or electronic format. This includes safety data, laboratory data (both clinical and immunological) and outcome data. Data will be managed and stored in MACRO® database, a GCP-compliant electronic data capture system. A study data management plan will outline detailed procedures for data capture, storage, curation and preservation.

The documentation, data and all other information generated will be held in strict confidence. Only authorized, trained study staff will have access to study records. The investigators will permit authorized representatives of the sponsor, ethical committee(s), regulatory authorities (if applicable), authorized representative of sponsor, and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. No information concerning the study or the data will be released to unauthorised third parties, without prior written approval of the Sponsor.

B6.5 Data analysis

The safety of the CHMI will be assessed by descriptive analysis of the frequency, incidence and nature of adverse events and serious adverse events arising during the study. Since this is a feasibility study conducted in 2 volunteers per dosing group, formal statistical hypothesis testing will not be used for most analyses due to the limited sample size, and only a brief Statistical Analysis Plan (SAP) will be developed and finalized prior to database lock.

B7. DATA AND SPECIMEN STORAGE AND/OR SHARING

B7.1 Management of specimen/data archiving and of left-over samples

Specimen storage

With the volunteers' informed consent, any leftover cells and serum will be stored for 10 years in the FTM/MORU Sample Management System for future immunological and genetic analysis of malaria-specific responses.

Data handling and record keeping

Medical records and study documentation/data will be kept at study site for approximately 5 years after study completion. Data will be fully anonymized and stored indefinitely in a secure database.

B7.2 Specimen/data sharing plan

All data used for analysis will be de-identified; personal identifying information such as names and telephone numbers will not be used for analysis. Files containing identifiable information will be stored separate from other study data, in secure locations. Only the Sponsor's representative, Investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to these records. Photographs may be taken to be shown to other professional staff, used for educational purposes, or included in a scientific publication. Photographs will not include the volunteer's face. And the volunteer's written informed consent will be sought before photographs are taken. The photographs will be stored as confidential records, as above.

De-identified data and results from blood analyses stored in our database may be shared with other researchers to use in the future.

PART C: ETHICAL CONSIDERATION (describe only the responsibilities of the PI)

C1. SIGNIFICANCE OF THE STUDY

This study will be a proof of feasibility and safety of controlled blood-stage human *P. vivax* infection using cryopreserved inoculum. The optimum dose of inoculum determined in this study will be used in future CHMI and malaria vaccine studies. This study will also provide data on the immune response to malaria infection, gametocytemia and transmissibility of the gametocytes in volunteers.

C2. BALANCE OF RISK AND BENEFIT

C2.1 Risk of the study

This section describes the foreseen risks to the volunteer including risk related to:

Phlebotomy

The maximum volume of blood drawn over the study period should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venipuncture, which will not be documented as AEs if they occur.

Inoculation

This controlled blood-stage challenge will involve a transfusion of a blood inoculum. As with any transfusion, there are risks of blood-borne infections and allergic reaction to blood products. The risk of blood-borne infection was reduced during the collection of blood inoculum in MIST 1 by extensive screening for blood-borne infection in both the source patients and blood donation volunteers. Serious allergic reactions including anaphylaxis have not been seen in challenge studies to date, but for safety reason volunteers will be inoculated in an area where physicians and a defibrillator are immediately available.

As the inoculum is prepared from packed red cells, the volunteers have a risk of developing a transfusion reaction. This risk has been minimized by preparing the inoculum from donors with blood group O (universal donors), and the volume of blood to be used (0.5 mL packed red cells) is much smaller than that given in transfusion of one unit packed red cells (470 mL). Nevertheless, the volunteers will be monitored for this possibility during the first hour after the administration of the inoculum.

Plasmodium vivax infection

Base on previous studies, volunteers are likely to develop symptomatic malaria infection following challenge [18]. The volunteers will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the study schedule. We will admit the volunteers to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University for close clinical monitoring, treatment (antimalarials) and symptomatic relief. A very small proportion of volunteers in previous *P. vivax* blood-stage and sporozoite-stage challenge studies have temporarily required intravenous fluid therapy for nausea and vomiting prior to treatment.

C2.2 Preventive and alleviative measures for risk

Phlebotomy

Participants' blood samples will be collected by well - trained medical personnel with aseptic technique to prevent infection or complications.

Plasmodium vivax infection

Volunteers will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the intensive follow-up schedule.

As described above, the volunteers will be hospitalized in the Hospital for Tropical Medicine, Faculty of Tropical Medicine, Mahidol University for close clinical monitoring, antimalarial treatment and symptomatic relief (e.g. fluids, analgesia and/or anti-emetics) until parasitaemia becomes negative. The treatment algorithm as well as the hospital guideline will be fully reviewed and strictly followed. The hospital will be officially informed prior to study initiation to ensure all the required medical facilities are in place. Clinical malaria expert at the Faculty of Tropical Medicine will be officially informed prior to study initiation. The medical monitors will be reachable throughout the whole study process.

In addition, this blood-stage inoculation will cause *P. vivax* infection without liver-stage parasites, thus removing the risk of relapse.

COVID-19

Risk mitigation steps will be taken for all face to face research during the COVID-19 pandemic, following local guidance (Hospital for Tropical Diseases).

The subject going missing post-challenge

The volunteers will be inpatients for entirety of the challenge phase, from the day before challenge until the completion of antimalarial treatment. It is unlikely that a volunteer will go absent during hospitalization (before antimalarial treatment is completed).

C2.3 Benefits of the study

The expected benefit is to provide information about the feasibility and safety of and immune response to *P. vivax* infection through experimental inoculation of cryopreserved infected erythrocytes, and on the optimal inoculation dose for future testing of pre-erythrocytic candidate vaccines. All of these will benefit patients with malaria infections in the future, and potentially lead to the development of a vaccine to prevent infections altogether. However, there are no direct benefits to the participants taking part in this study.

C3. SAFETY

C3.1 Safety experience from previous CHMI studies

Following a collaborative consensus process involving investigators from the USMMVP, Sanaria, University of Maryland, University of Oxford, RUNMC, the Seattle Biomedical Research Institute and the KEMRI-Wellcome Kilifi Research Programme, a consensus document; "Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials" was developed. This provides a comprehensive guide to the appropriate conduct of controlled human malaria infection studies [22]. Although there remain minor differences between centres in follow-up procedures in controlled human malaria infection trial conduct, there is consensus on the following key points.

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, pregnancy test for female volunteers.
- Follow-up visits should be scheduled at least once daily, but may increase in frequency to twice daily, starting at day 5 post-sporozoite challenge. At all visits volunteers should be questioned about the occurrence of adverse events (AEs) and use of medication.
- Grading and reporting of adverse events should be performed using international and local guidelines. It should be noted that the occurrence of a low frequency of grade 3 severe adverse events, of short duration, and with no long-term sequelae, is not unexpected in control human challenge studies. A minority of those challenged are known to experience grade 3 systemic adverse events and this fact should be included in the informed consent form.
- Vital signs should be recorded at least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every volunteer must receive every dose of antimalarial therapy. In some settings fully directly observed treatment will be essential. Where directly observed treatment is not used, investigators must follow volunteers closely to ensure compliance with the treatment regimen.

- After challenge, all volunteers should be followed until they have completely finished antimalarial treatment.
- Volunteers should be evaluated at least two weeks after finishing treatment.
- A local safety monitor and an independent safety monitoring committee should be established to act as independent experts in evaluating adverse events. The safety monitor or monitoring committee may advise the investigators on initiating anti-malarial treatment for a specific volunteer or volunteer group. While safety monitoring committees are not a requirement for Phase I trials, they should be considered a requirement for challenge trials which have an efficacy component and which have major potential safety concerns.

Volunteer safety is of paramount importance, and we will follow these guidelines. The following measures are in place to safeguard volunteer safety:

- Volunteers will only be enrolled in the study if investigator judges this is appropriate.

- Volunteers' understanding of the trial information will be tested **by means of a questionnaire** at screening. This provides further confidence that fully informed consent has been obtained.

- Before challenge, full contact details for each volunteer will be documented, including home address and mobile telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the volunteers are easily contactable. Home and work landline telephone numbers where available and next-of-kin address and telephone numbers will also be documented. Volunteers must also provide the Investigators with the name and 24 hour telephone number of a close friend(s), relative(s) or housemate(s) who live nearby and who will be kept informed of their whereabouts for the duration of the study.

- The volunteers will be hospitalized throughout the challenge processes until clinically recovered **AND** the completion of the antimalarial course (chloroquine) **AND** until two consecutive negative blood films.

- Volunteers will be able to contact a medically qualified member of the study team 24 hours a day throughout the study period.

Expectations of volunteer events following blood stage challenge

This part of the protocol aims to clearly review the events related to experience from the previous control human challenge studies in order to foresee the possible event(s) after the challenge that we may have to prepare to encounter within this study.

- According to references No. 18-21 and personal communication with other investigators, all volunteers in *P. vivax* blood stage challenge studies developed infection within 3 weeks following the challenge. Most of AEs were mild to moderate grade and all were transient. No serious adverse events were reported.

C3.2 Safety Monitoring

C3.2.1. Medical Monitor

A Medical Monitor group, representing the Sponsor, will be appointed for oversight of safety in this clinical study. The Medical Monitor group will be responsible for safety assessments. The monitor will review the study prior to initiation and will be available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. The medical monitor does not have direct involvement in the conduct of the study. All serious adverse events will be reported to the medical monitor within 24 hours of becoming aware of the event. The medical monitor group is responsible for the review of the safety data and communicate with the PI and/or the DSMB, as appropriate.

The appointed medical monitor for the MIST 2 study is Dr. Lorenz Von Seidlein M.D.

C3.2.2. Data and Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) will review the study prior to initiation; review the interim safety data reports; and review all serious adverse events (SAEs) according to DSMB charter. The Board may convene additional reviews if deemed necessary, on review of the safety data, as sent, periodically by the medical monitor. All SAEs will be reported by the site principal investigator to the DSMB at the same time as they are submitted to the ethics committees. The Co-Principal Investigator(s) will notify the Board and obtain a recommendation concerning continuation, modification, or termination of the study. The site principal investigator will submit written DSMB summary reports with recommendations to the ethics committees(s). The roles and responsibilities of the DSMB will be formalised in a charter agreed with the members of the DSMB.

The DSMB will be independent of the Clinical Trials and Research Governance, Oxford University (Sponsor) and MIST programme team. The DSMB is charged with monitoring and evaluating the clinical data generated by this study, with a focus on safety, in and independent and objective manner. The main roles and responsibilities of the DSMB, DSMB procedures, and the DSMB meeting schedule are defined in the Data and Safety Monitoring Board Charter for the Malaria Infection Study in Thailand (MIST) Programme.

C4. CONSIDERATION FOR VULNERABLE RESEARCH PARTICIPANTS

Check whether your study involves any of the following vulnerable research participants.

- ☐ Prisoners
- ☐ Pregnant women
- ☐ Mentally ill persons
- ☐ Cancer or terminally ill patients
- ☐ Neonates/infants/children (aged <18)
- ☐ HIV/AIDS patients
- ☐ Institutionalized persons e.g. military, students, etc.
- ☐ Others (please specify)……
- ☑ Not applicable

C5. INFORMED CONSENT ISSUES

C5.1. Informed consent process

The informed consent process: The investigator will explain the purpose of the study with the help of and consistent with the Participant Information Sheet (PIS) prior to any study related procedures being undertaken. The volunteer must personally sign and date the latest approved version of the informed consent form (ICF) before any study specific procedures are performed.

The information sheet and informed consent form will be explained to the volunteers detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. The aims of the study and all procedures to be carried out will be explained. **It will be emphasised that there is no direct benefit from participating the trial**.

- It will be clearly stated that participation is entirely voluntary and that refusing to participate will not involve any penalty or affect the volunteers' right to receive standard medical care.

- It will also be emphasized that if they do consent to participate and are enrolled, that they are free to withdraw from the study at any time, for any reason, without any penalty or prejudice to future care, and with no obligation to give the reason for withdrawal.

The volunteer will have the opportunity to question the investigator, or other independent parties to decide whether or not they will participate in the study.

If they do decide to participate, volunteers will be asked to **complete a questionnaire testing their understanding of the trial before signing the consent.** This helps to ensure that individuals understand the trial sufficiently to give informed consent. After the volunteer answers all questions in the questionnaire correctly, they will be asked to sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the investigator's site file and retained at the study site. These forms will also be signed and dated by the investigator. Volunteers who fail to answer all questions correctly on their first attempt will be allowed to re-take the questionnaire following further discussion with the investigator. Only the subject who able to subsequently answer all questions in the questionnaire correctly will be asked to give the consent and will be screened for the trial. Only 2 attempts will be permitted in total.

Hospital consent for HIV test: This will be obtained prior to counselling and HIV test using the standard HIV consent from of Hospital for Tropical Diseases. Pre and post counselling for HIV screening and reporting will be conducted with the support of physician from the Hospital for Tropical Diseases. In case a volunteer is found to be HIV positive, follow up measures including but not limited to providing counselling and treatment according to standard hospital procedure will be arranged through a physician from the Hospital for Tropical Diseases.

C5.2 Informed consent documentation

Age	**Informed Consent**	**Participant Information Sheet**
20 years and over (Adult)	ICF for Adult Version 6.0, dated 27 April 2022	PIS for Adult Version 6.0, dated 27 April 2022
	ICF for Data Sharing/ Data and Leftover Specimen Storage from Current Study for Future Use Version 6.0 dated 27 April 2022	
	ICF for Leftover Specimen Storage from Current Study for future genetic analysis Version 6.0 dated 27 April 2022	
20 years and over (Adult)	HIV Pre-test counseling form Version 1.0, dated 1 October 2009	

C5.3 Compensation for research participants

☑ Yes, please provide details:

Volunteers will be compensated for their time and for the inconvenience caused by procedures as below (Table 8). This amount of compensation is calculated based on the cost of living in Bangkok for time and traveling. According to the consensus recommended by the controlled human challenge study group meeting in 2015, compensation specifically for risk was also advised (Bambery B, Selgelid M, Weijer C, et al., Ethical criteria for human challenge studies in infectious disease. Public Health Ethics. 2016. 9; 92-103.)

Table 8*:* Estimated compensation amounts.

Activity	Compensation (THB)	Number of visits	Total (THB)
1. Screening visit (D-14)	2,000	1	2,000
2. Admission per night	2,000	Around x 18 nights	36,000 (estimated)
3. D_{Rx7}	1,500	1	1,500
4. D_{Rx28}	1,500	1	1,500
5. D_{Rx60}	1,500	1	1,500
6. D_{Rx90}	1,500	1	1,500
7. D_{Rx180}	1,500	1	1,500
8. $D_{Rx\ 1\ yr.}$	1,500	1	1,500

Time in Trial (approx.)	Maximum no. of visits	Maximum volume of blood taken[1] (mL)	Total compensation amount (THB)
1 year	8	365 mL	47,000

Remark:

[1]Maximum volume of blood taken is 365mL in case volunteer received antimalarial treatment on day 16.

In case a volunteer has to come for extra visit(s), they will be compensated for their time and for the inconvenience with 1,000 THB per day.

☐ No, please provide reasons: ……..……

C5.4 Responsible and contact persons

☑ Person(s) responsible for payment for treatment of complications and adverse effects

☑ Person(s) including doctor(s) and/or contact address(es) and telephone number(s) for emergency use

1. **Dr. Borimas Hanboonkunupakarn**, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 354-9100 ext. 3160, Mobile (086) 970-5705
2. **Dr. Podjanee Jittamala,** Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 306-9157, Mobile (081) 956-3371.
3. **Dr. Kittiyod Poovorawan**, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Tel. (02) 354-9100 ext. 1435, Mobile (083) 149-6864

PART D: APPENDIX A

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APPENDIX B: SUBMISSION PACKAGE INCLUDES

No.	Submitted Documents
1.	Research Proposal Submission Form for a study involving human subject enrollment WITH specimen collection (FTM ECF-033-RR)
2.	Informed Consent Form and Participant Information Sheet
3.	Informed Consent Form for Data Sharing/ Data and Leftover Specimen Storage from Current Study for Future Use
4.	Informed Consent Form for Leftover Specimen Storage from Current Study for future genetic analysis
5.	HIV Pre-test Counseling Form
6.	Questionnaire to evaluate participant's understanding of the study
7.	Recruitment material (flyer)
8.	Diary card
9.	Screening Form
10.	Case Record Form

APPENDIX C: SAFETY TERMS AND ADMINISTRATIVE INFORMATION

1. SAFETY DETECTION, ASSESSMENT, DOCUMENTATION AND REPORTING

The investigators and designated site staff is/are responsible for the detection, assessment, documentation and reporting of events meeting the criteria and definition of an adverse event (AE), or serious adverse event (SAE) as provided in this Research Proposal Submission Form. Volunteers will be instructed to contact the investigator immediately should the volunteer manifest any signs or symptoms they perceive.

1.1 Definitions

1.1.1 Adverse Event (AE):

An AE is any untoward medical occurrences in a volunteer, which may occur during or after administration of a study intervention (in this case, vivax parasite challenge) and does not necessarily, have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention. Any events occurring between screening and D-1 will be considered as baseline/, pre-existing conditions. This information will be recorded in the medical records.

1.1.2 Serious Adverse Event (SAE):

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death (i.e. results in death from any cause at any time).
- Life-threatening event (i.e. the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Transfer of inpatient care to the intensive care unit, if the Investigators assess that a higher level of intervention and intense monitoring is required to manage symptoms following controlled human malaria infection, or drugs (over and above what can be provided by the Investigators in research bay). Hospitalization (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a SAE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

1.2 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events:

In absence of a diagnosis, clinically abnormal laboratory findings (e.g. clinical chemistry, haematology, and urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as an AE or SAE if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

In addition, any COVID-19 infection in volunteers acquired during the in-patient stay following challenge will be considered an SAE and COVID-19 treatment will be provided to the volunteers free of charge. If volunteers have to stay in the hospital for SAE, admission compensation at 2,000 THB per night will be provided to the volunteers.

1.2.1. Causality assessment

The investigator is obligated to assess the relationship between study procedure and/or antimalarial medications and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study procedure and antimalarial medications will be considered and investigated. The relationship of the adverse event with the study procedures will be categorized as unrelated, unlikely to be related, possibly related, probably related or definitely related (Table C1). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The delegated clinician will use clinical judgment to determine the relationship.

Table C1: Guidelines for assessing the relationship of study intervention to an AE

0	Unrelated	No temporal relationship to study intervention and Alternate aetiology (clinical state, environmental or other interventions); and Does not follow known pattern of response to CHMI, blood donation or drug.

1	Unlikely to be related	Unlikely temporal relationship to study intervention and Alternate aetiology likely (clinical state, environmental or other interventions); and Does not follow known typical or plausible pattern of response to challenge, blood donation or drug.
2	Possibly related	Reasonable temporal relationship to study intervention; or Event not readily produced by clinical state, environmental or other interventions; or Similar pattern of response to that seen with previous challenge, blood donation or similar drug.
3	Probably related	Reasonable temporal relationship to study intervention; and Event not readily produced by clinical state, environment, or other interventions; or Known pattern of response seen with previous challenge, blood donation or drug.
4	Definitely related	Reasonable temporal relationship to study intervention; and Event not readily produced by clinical state, environment, or other interventions; and Known pattern of response seen with previous challenge, blood donation or drug.

1.2.2. Assessment of AE intensity

Each adverse event will be graded by the investigator and designated study staff according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

In the rare case that an adverse event is not graded in the CTCAE, then that event should be graded as follows:

Grade 1: Mild AE
Grade 2: Moderate AE
Grade 3: Severe AE
Grade 4: Life-threatening or disabling AE
Grade 5: Death related to AE

All AE/SAE will be collected throughout the first 3 month after challenge or until a satisfactory resolution occurs. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). In cases where the volunteer has to do AE self-detection and self-assessment, a simple grading system for the self-assessment on the diary card will be used (Table C2).

Table C2: Severity grading criteria for the self-assessment for AEs.

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; may require medical intervention/therapy

1.2.3. Assessment of outcomes

The investigator will assess the outcome of all AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

During the inpatient phase, the adverse events (including all SAEs) will be assessed by the investigator and the delegated study staff.

While the volunteer is followed as an outpatient, a diary card will be provided, and the volunteer will be instructed how to self-assess the cause and severity of AEs. This self-assessment symptom diary will be checked, discussed, collected and recorded at each clinic visit.

1.3 Reporting procedures

For SAEs

In order to comply with current regulations on serious adverse event reporting to Ethics and regulatory authorities (if applicable), the event will be documented accurately and notification deadlines respected.

SAEs will be reported to the medical monitor immediately (within 24 hours) of the Investigators' being aware of their occurrence.

SAEs will also be reported to ethics committees, the Data and Safety Monitoring Board (DSMB), and the regulatory authority (if applicable), in accordance with reporting requirements and according to required timelines.

1.4 Events or outcomes not qualifying as adverse events or serious adverse events

Pregnancy

Volunteers are informed that for the safety of volunteer and their child, pregnancy is prohibited until 3 months after the challenge. Female study volunteers are asked to use appropriate contraceptive methods to prevent pregnancy while they participate in the study for 3 months after the injection of malaria infected erythrocytes.

Contraceptive methods include:

- Established use of oral, injected or implanted hormonal contraceptives
- Intrauterine Device or Intrauterine System
- Barrier methods (condoms or diaphragm with additional spermicide)
- Male sterilisation and female sterilisation (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate)
- True abstinence, when this is in line with the preferred and usual lifestyle of the volunteer. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Female volunteers will be tested for pregnancy immediately prior to challenge and during follow up as indicated in the schedule of procedures (Tables 4 and 5).

Should a volunteer become pregnant during the trial, she will be treated with antimalarials immediately and will be withdrawn from the study. We will not routinely perform venepuncture on such volunteers, other than blood films to check that any parasitaemia has been cleared by the antimalarial treatment. With the volunteer's permission she shall be followed up until pregnancy outcome. The management of any volunteer found to be pregnant at any time after challenge up to the point of malaria treatment will be discussed with the on-call infectious diseases consultants at Faculty of Tropical Medicine and the Mahidol Oxford Tropical Medicine Research Unit, including advice on antimalarial drug choice.

Should a volunteer become pregnant after receiving antimalarial treatment (but prior to the end of the study), they shall be discontinued from the study as soon as we have confirmed that their parasitaemia has cleared. The volunteer will still be followed up until 3 months after challenge.
During pregnancy the following should always be considered as an SAE:

- Spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
- Ectopic and molar pregnancy
- Stillbirth (intrauterine death of fetus after 22 weeks of gestation).
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).

- Any congenital anomaly or birth defect identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

However, all pregnancies occurring in participants within 3 months of challenge will be considered a SAE.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study procedures will be reported. While the investigator is not obligated to actively seek this information from former study volunteers, he/she may learn of a pregnancy through spontaneous reporting.

All pregnancies occurring during the study will be reported to the DSMB.

1.5 Safety monitoring

Monitoring

Monitoring will be performed using an established Monitoring Plan. Independent monitoring will be performed by Clinical Trials Supporting Group (CTSG), MORU. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol and Good Clinical Practice (GCP). The investigators will provide direct access to all trial related source data/documents and reports for the purpose of auditing by the Sponsor and inspection by local and regulatory authorities.

2. RANDOMIZATION

This will be a double-blinded design. Participants will be recruited and randomised in blocks of size 8 to one of the 4 inoculation dilutions using a computer program: whole dose blood-stage inoculum (neat) or 1:5 or 1:10 or 1:20 dilution blood-stage inoculum. Thus, by the end of each block, each of the inoculation dilution group will have 2 participants i.e. in the ratio 2:2:2:2.

The randomization lists, will be prepared by MORU statistician and the randomization envelopes will be prepared by Clinical Trials Support Group (CTSG)/MORU.

For each inoculation dilution group, 2 randomization numbers will be generated in blocks of size 8 so, for the 4-dose inoculation arms in a ratio of 2:2:2:2 as follows:

1. whole dose blood-stage inoculum (neat)
2. 1:5 dilution blood-stage inoculum
3. 1:10 dilution blood-stage inoculum
4. 1:20 dilution blood-stage inoculum

Study volunteers will be assigned a randomization envelope, and thus will be randomly allocated to inoculation arm 1, 2, 3, or 4. This is a double-blinded study; volunteers, clinical investigators, and study staff who perform laboratory study endpoint will be blinded to inoculation arm allocation.

Only MVRU staff who prepare the inoculum and CTSG statistical staff will know the concentration of each inoculum and these individuals will not be involved in providing volunteer care, investigation, and performing laboratory study endpoint. In addition, these individuals will not be involved in any other study assessment and evaluation.

3. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures and prespecified monitoring plan, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the current approved protocol, standard operating procedures, ICH GCP and relevant regulations.

The Sponsor and authorised individuals may carry out audit to ensure compliance with the protocol, standard operating procedures, ICH GCP and relevant regulations. The study audits will be managed by an independent function according to standard operating procedures a prespecified audit plan.

GCP inspections may also be undertaken by the regulatory authority or ethical committee(s) to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

4. ETHICS

Ethical approval will be sought prior to commencing the study through the relevant Research Ethics Committees. Indemnity for the trial will be provided by the University of Oxford. SAEs will be reported to the medical monitor, DSMB, and the ethics committees. GCP certificate will be obtained by all staff/investigators prior to commencing the studies.

The Investigators will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki 2013. The trial will adhere to the ICH GCP E6 (R2).

The protocol, informed consent form, participant information sheet, and other written participant information/materials and Advertisement will be submitted to appropriate Research Ethics Committees (RECs), and regulatory authorities (if applicable) for written approval. The Principal Investigator (PI) will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents. The Investigator will notify deviations from the protocol or SAEs occurring at the site to REC(s) in accordance with procedures.
The principal investigator shall submit a report once a year throughout the study, or on request, to the ethic committees. In addition, an End of Study notification and final report will be submitted to the ethic committees.

5. SPONSOR: University of Oxford

Contact information: University Offices, Wellington Square, Oxford, OX1 2JD, United Kingdom

The study sponsor has a role in the design of the study; collection of data; management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

6. FUNDER

The funder, the Wellcome Trust, had no role in the design of the study. The funder will not have any role in its execution, analyses, and interpretation of data or decision to submit results.

7. TRIAL COMMITTEES

Programme Steering Committee (PSC): The role of PSC is to provide overall supervision for the project on behalf of the Project Sponsor and Project Funder and to ensure that the project is conducted to the rigorous standards set out in the Research Governance Framework for Health and Social Care, and in the Guidelines for Good Clinical Practice. The composition and roles and responsibilities of the PSC, and the PSC meeting schedule, are defined in the PSC Terms of Reference.

8. INSURANCE

The University of Oxford has a specialist insurance policy in place - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any volunteer suffering harm as a result of their involvement in the research.

9. PUBLICATION POLICY

All Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged.

The trial results for each volunteer will be communicated individually to that individual to inform his/her health history.

APPENDIX D: LIST OF ABBREVIATIONS

AE	adverse event
β-HCG	beta–Human Chorionic Gonadotropin
BP	blood pressure
BT	body temperature
BUN	blood urea nitrogen
CBC	complete blood count
CHIK	chikungunya
CHMI	controlled human malaria infection
CMV	cytomegalovirus
Cr	creatinine
CRF	case record form
CRO	Contract Research Organization
CSP	circumsporozoite
CTCAE	Common Terminology Criteria for Adverse Events
CTSG	Clinical Trials Supporting Group
CYP2D6	enzyme predicting primaquine metabolism (p450 enzyme)
DARC	Duffy antigen receptor for chemokines
DBP	Duffy binding protein
DEN	Dengue
DFA	Direct Feeding Assay
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
ELISA	Enzyme-linked Immunosorbent Assay
FBS	fasting blood sugar
FTM	Faculty of Tropical Medicine
FTMCTU	Clinical Therapeutics Unit (healthy volunteer ward), Faculty of Tropical Medicine
FTMEC	Ethics Committee, Faculty of Tropical Medicine
G6PD	glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HTLV	Human T cell Lymphotrophic virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
JE	Japanese Encephalitis
KEMRI	Kenya Medical Research Institute

LDL	low density lipoprotein
LFT	liver function test
MFA	membrane feeding assay
MORU	Mahidol Oxford Tropical Medicine Research Unit
MVRU	Mahidol Vivax Research Unit
OxTREC	Oxford Tropical Research Ethics Committee
PCR	polymerase chain reaction
P. falciparum	*Plasmodium falciparum*
PI	Principal Investigator
PIS	Participant Information Sheet
PMR	parasite multiplication rate
PQ	primaquine
PR	pulse rate
P. vivax	*Plasmodium vivax*
PvCSP	*P. vivax* circumsporozoite
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RR	respiratory rate
RUNMC	Radboud University Nijmegen Medical Centre
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
TMDR	Tropical Medicine Diagnostic Reference Laboratory
QIMR	Queensland Institute of Medical Research
ULN	upper limit of normal
USMMVP	U.S. Military Malaria Vaccine Program
WHO	World Health Organization
WI	Work Instruction
ZIK	Zika virus

APPENDIX E: AMENDMENT HISTORY

Amendment No.	Proposal Version No.	Date issued	Author(s) of changes	Details of Changes made
1	v.4.0	1 November 2021	Salintip Chanchaivorawith, MPH	1.The list of investigators is updated. 2.PIS/ICF documentation is updated.
2	v.5.0	7 February 2022	Salintip Chanchaivorawith, MPH	1.For inclusion criteria, COVID-19 vaccination at least two doses of a vaccine(s) is included. 2.For exclusion criteria, clarifying about COVID-19 testing that must be diagnosed by PCR (nasopharyngeal swab). 3.To include EBV and CMV serology testing in screening visit 3 (day -1) and during 2-4 weeks after the inoculation. 4.To include COVID-19 serology Point-of-Care Test (POCT) in screening visit 3 (day -1) and day -14 after the inoculation. 5.Administrative procedures: one withdrawal criteria/discontinuation criteria about current COVID-19 infection during participant's admission in the hospital (after the inoculation) is added. 6.Administrative procedure: to update criteria of COVID-19 testing during participant's admission in the hospital.

3	V6.0	27 April 2022	Assist. Professor Borimas Hanboonkunupakarn	1) To remove Hemoglobin testing from screening1 2) To change COVID-19 serology POC test to COVID-19 serology 3) To specify 2nd COVID-19 serology test at Day discharge 4) To revise blood collected volume according to the new laboratory requirement and new tests 5) To revise laboratories and permission to perform lab test (s) at other validated 6) laboratories 7) Administrative changes and some typing errors